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Short Communication

Low seroprevalence of cryptococcal antigenaemia in patients with advanced HIV infection enrolling in an antiretroviral programme in Ghana

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Summary

OBJECTIVES To determine the prevalence of cryptococcal antigenaemia in a clinic population with advanced HIV infection, with a view to giving antifungal therapy to those testing positive.
METHODS Serum samples from adults with CD4 count <100 cells/mm^3 presenting to a large HIV clinic in Kumasi, Ghana, were tested retrospectively for cryptococcal antigenaemia using a latex agglutination assay, and clinical and demographic data extracted from case notes.
RESULTS Of 92 samples tested, two were positive thus giving a prevalence of 2% (95% CI, 0–5.2%).
CONCLUSIONS The prevalence of cryptococcal antigenaemia in patients with advanced HIV infection enrolling in an antiretroviral programme appears to be low in Kumasi, suggesting that the value of routine testing of outpatients diagnosed with advanced HIV infection may be limited in this population.

keywords Cryptococcus, HIV, Africa, epidemiology, cryptococcal antigen

Introduction

Cryptococcal meningitis (CM), a fungal infection caused by *Cryptoccoccus neoformans*, has emerged as a leading cause of mortality in HIV-infected patients in the developing world (Okongo *et al.* 1998; Corbett *et al.* 2002; French *et al.* 2002; Castelnuovo *et al.* 2009). In sub-Saharan Africa, CM accounts for 20–50% of this early mortality, with an estimated half million of AIDS-related deaths per year (Park *et al.* 2009). Studies in Uganda and Tanzania have shown that 80–90% of patients with CM had CD4 counts of <100 cells/mm^3 (French *et al.* 2002; Kisenge *et al.* 2007). Cryptococcal antigenaemia is detectable a median of 22 days before the onset of symptoms (French *et al.* 2002) and has been shown to be 100% sensitive for predicting the development of CM in the first year of antiretroviral therapy (ART) (Jarvis *et al.* 2009), as well as being associated with both CM and mortality in two Ugandan studies (Castelnuovo *et al.* 2009; Meya *et al.* 2010). Cryptococcal antigen (CRAG) tests on serum are highly sensitive and specific and have been validated for use in the HIV-infected population (Temstet *et al.* 1992; Tanner *et al.* 1994; Lara-Peredo *et al.* 2000). Screening for subclinical or asymptomatic infection by a serum CRAG assay in patients with advanced HIV infection, and giving antifungal therapy to those testing positive, may prevent the development of CM; the use of fluconazole in patients with asymptomatic cryptococcal antigenaemia was associated with an odds ratio of survival of 26.2 in one study (Meya *et al.* 2010).

The prevalence of cryptococcal antigenaemia in patients presenting with advanced HIV infection has been estimated to be between 7% and 13% in Uganda, South Africa and Thailand (Tassie *et al.* 2003; Jarvis *et al.* 2009; Meya *et al.* 2010; Pongsai *et al.* 2010), but is still unknown in many parts of Africa, including West Africa. The only population-based estimates of the burden of cryptococcal disease in this region are based on data extrapolated from provider-based studies (Park *et al.* 2009), and CM is infrequently diagnosed in patients admitted with advanced HIV infection in Kumasi. We conducted a retrospective study in a large government hospital in Ghana on stored

serum samples to estimate the prevalence of cryptococcal antigenaemia in outpatients presenting with advanced HIV infection and to study the effect of cryptococcal antigenaemia in terms of short- to medium-term mortality, response to ART and clinical features of subsequent cryptococcal disease.

The approval of the Committee on Human Research and Ethics at KNUST was obtained for this study. Serum samples of adult HIV-positive patients, diagnosed from April 2008 to May 2009 and who attended the HIV clinic at Komfo Anokye Teaching Hospital (KATH), were collected and stored. Patients who had an initial baseline CD4 < 100 cells/mm^3 and had a serum sample taken within 6 months of diagnosis (or within 3 months of starting ART) were eligible for inclusion in this study. Demographic data, ART (if applicable), body mass index (BMI), CD4 counts, HIV stage and documented clinical events or clinical signs suspicious of cryptococcal disease were recorded. Stored serum samples were tested using Latex-Cryptococcus Antigen Detection System (Immuno-Mycologics, USA), according to the manufacturer's instructions, both in Kumasi and later in London (for confirmation), with CRAG titres estimated on positive samples.

Ninety-two patients with a serum sample available were eligible for the study, representing over 80% of all patients who presented with a CD4 < 100 cells/mm^3 in this time period. The median time from a positive HIV test to obtaining the sample used for CRAG testing was 2 days (IQR 1–6), with 78% of samples obtained within 1 week of diagnosis of HIV infection. Only two serum samples were positive for cryptococcal antigenaemia, one positive up to a dilution of 1:16 and the other only positive on neat serum. The prevalence of antigenaemia in this population is therefore estimated to be 2% (95% CI, 0–5.2). The first patient presented to the clinic in 2008 with a baseline CD4 count of 31 cells/mm^3. The patient was a 58-year-old man with a BMI of 16 and WHO Stage 1 disease at diagnosis. There were no clinical symptoms or features of cryptococcal infection at presentation or follow-up. He defaulted follow-up after his second clinic visit and did not start ART. The second patient with positive CRAG on neat serum only, so not a clear positive, was a 40-year-old woman who presented to the clinic in 2008 with a CD4 count of 4 cells/mm^3 and pulmonary tuberculosis. She was started on ART 6 months after diagnosis and has not defaulted from follow-up. Because of the low prevalence rate of cryptococcal antigenaemia, no meaningful comparison between demographic and clinical features of the CRAG-positive and CRAG-negative patients was feasible.

Table 1 shows the baseline characteristics of the study population. Demographic and other features of this cohort were similar to the overall group of patients presenting with a CD4 count below 100 cells/mm^3. The median CD4 count at diagnosis was 28 cells/mm^3 (IQ range 8–54), whilst most patients were classified as WHO Stage 3. Comprehensive clinical data were available for 56 patients, of whom 28 presented with only one symptom at diagnosis and 28 with two or more when first seen in clinic. Symptoms recorded were almost entirely non-specific e.g. weight loss. Fifty-three patients defaulted follow-up in clinic, mostly within the first 6 months of follow-up, and although precise data on mortality were not available (many patients travelled long distances to attend clinic), it is likely many of these patients died shortly after diagnosis. In this group, only 18 were started on ART prior to defaulting, with a median delay of 36 days (IQ range 21–58) from diagnosis. There were 39 patients who continued to attend clinic, and who were all started on ART with a median delay of 33 days from diagnosis (IQ range 28–50). Interestingly, a high proportion presented with a WHO stage of 1/2 despite having a CD4 < 100 cells/mm^3.

This study has demonstrated a low prevalence of cryptococcal antigenaemia in outpatients presenting with

Table 1. Baseline characteristics of study population

	Characteristic	Interquartile range
Gender		
Male (%)	38 (41%)	
Female (%)	54 (59%)	
Age	40	33–46
Body Mass Index (kg/m^2)	20.2	19.7–21.2
CD4 count (cells/mm^3)	28	8–54
TB diagnosed at presentation	13 (14%)	
WHO stage (*N*)	58	
I	12 (21%)	
II	11 (19%)	
III	26 (45%)	
IV	9 (15%)	
Non-defaulters		
N	39 (42%)	
Time (days) to starting antiretroviral therapy (ART) from diagnosis	33	28–50
Defaulters		
N	53 (58%)	
Median number of days of follow-up	167	8–374
Number started on ART prior to defaulting	18 (34%)	
Time (days) to starting ART from diagnosis	36	21–58

Median values shown unless stated otherwise.

advanced HIV infection in Ghana. If the true prevalence in this population is significantly lower than that observed in other populations in sub-Saharan Africa (given the upper 95% confidence limit of 5.2% may overlap with the lower limit from other studies), it remains unclear why the prevalence is lower. Although the sample size tested in this cohort was smaller than the three other populations studied in Uganda and South Africa (Tassie *et al.* 2003; Jarvis *et al.* 2009; Meya *et al.* 2010), the mean CD4 count in our cohort was substantially lower, which makes the apparent lower prevalence even more surprising. One possibility to explain this difference is selection bias, such that more patients in Kumasi with cryptococcal infection died before either being tested for HIV or attending the clinic. It remains unclear how significant this effect may have been given that referral processes and HIV testing rates appear to be similar in Ghana to Uganda and South Africa. However, it is possible that more patients with advanced HIV infection who were referred for a HIV test or admitted to hospital died of cryptococcal disease before having a HIV test and attending clinic. Unfortunately, as the CRAG test was not routinely available for patients admitted to the hospital, it was impossible to obtain data on rates of cryptococcal antigenaemia in patients admitted with advanced HIV infection or AIDS; clearly it would be useful to obtain these data in a future study.

The cost of preventing one death through CRAG screening (and fluconazole treatment) has been estimated to be $266 in a Ugandan cohort with a prevalence of asymptomatic antigenaemia of 13.5% (Meya *et al.* 2010). The cost effectiveness of such a screening strategy significantly diminishes once the prevalence of 'asymptomatic' antigenaemia falls below 5%, with an estimated cost of preventing one death being over $500 (Meya *et al.* 2010). Extrapolating the same cost-benefit analysis from Uganda to Ghana, and assuming costs of CRAG assays and fluconazole therapy are similar, the cost of preventing one death in Ghana, with a prevalence of antigenaemia between 0% and 5%, would range from $325 to over $3000. Hence even only selecting those with advanced HIV infection, if these costs are comparable, screening may not be economically viable. On the other hand, if costs of CRAG tests are lower than the $16/test assumed by Meya *et al.* (2010) or alternative strategies for reducing costs such as testing pooled samples are adopted, or the costs of antifungal drugs fall substantially, it may prove cost effective to screen patients with advanced HIV infection in Ghana.

This study also showed that most patients had to wait more than a month before starting ART, although in a proportion, the delay was due to their starting anti-tuberculous therapy. The high default rate (and presumed mortality) in this specific population suggests a need to review the HIV care programme in Ghana, to enable patients presenting with low CD4 counts to start ART sooner.

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